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Public Health Aspects of Water Fluoridation

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WHAT is the current status of fluoridation of communal water supplies for the partial prevention of dental caries? From the standpoint of the general public this may be answered by listing several items which are representative of the general happenings relating to fluoridation during 1953.

1. On January 26, 1953, the Canadian Medical Association and the Canadian Dental Association issued a joint statement supporting the fluoridation of communal water supplies for the partial prevention of dental caries.

2. In July, 1953, the American Dental Association released the following statement: "One out of every nine persons in the United States is routinely drinking water containing a beneficial fluoride content . . . 14,266,351 persons in 771 communities . . . a gain of 117 since January 1, 1953 . . . are served by fluoridation programs. Additionally, 3,570,021 individuals live in areas served by water supplies naturally containing desirable amounts of fluorides. The total of 17,836,372 persons represents 11 per cent of the nation."

3. On September 10, 1953, the president of the Canadian Medical Association made the statement that the "Canadian Medical Association is a little doubtful" about the process from a medical viewpoint. Pending the completion of a report, no stand for or against will be taken.

4. In the month of September two influential magazines published reports on fluoridation, stating that this measure is a "menace to life."

5. In September, 1953, the cities of Windsor, Ontario, and Assiniboia, Saskatchewan, approved fluoridation of city waters.

Such seemingly contradictory events must naturally confuse the public and create difficulties for the health teams doing promotional work, particularly the public health workers. Therefore it appears timely to review the basic facts regarding fluoridation of water supplies.

Historical Review

A suitable starting point in this discussion is to review briefly the early findings in this field. As early as 1807 it was established through the efforts of Morichini, Berzelius, Klaproth and Gay-Lussac that fluorine existed in fossil bones and teeth, and there was good evidence that it could be found in fresh bones and teeth (Cox, 1952). The association of fluorine with phosphorus was also noted. The crude analytical methods showed a far higher content of fluorine of bones and teeth than that which has been estimated with modern methods.

Investigators in about the first half of the 19th century established that fluorine was present in urine, blood, saliva, water, milk, and other miscellaneous fluids. The variability of the fluorine content of water was known in 1823; fluorine was noted in the London water supply in 1845. Thus the interest in fluorine is not without precedent.

At approximately the same time reports appeared in dental literature describing hypoplastic defects in the dentition (Magitot, 1867). In 1916, McKay and Black coined the phrase "mottled teeth" to describe a condition having the following characteristics: (a) restricted to certain areas, (b) absent from deciduous teeth, (c) ranging in severity from paper-white teeth and white flecks to black stained and pitted teeth, (d) absent in teeth formed outside the endemic area. It was soon observed that mottled enamel, "which is so obviously defective, is able to escape carious attack with not more than average frequency of incidence" (McKay, 1925). It was logically suggested that some trace element in food or water was the agent responsible for this condition.

The analysis of water indicated nothing but contradiction. McKay at this time wrote: "There are present, however, in waters, certain other elements of rarer varieties that exist only in traces, the determination of which requires much more elaborate technique . . . which are beyond the capacities of the ordinary chemical laboratories."

The discovery by Petrey in Churchill's laboratory in 1931 that fluoride concentration paralleled the severity of mottled enamel opened up the now famous epidemiological studies into the relationship of fluorine and dental health.

Natural Occurrence of Fluorine in Food and Water

Many different foods and most natural water supplies contain trace quantities of fluorine. Thus, under the usual conditions of living, minute quantities of fluorine are consumed by most individuals. The claim that addition of fluoride to water constitutes medication is, therefore, unjustified.

Food supplies approximately 0.2–0.3 mg. of fluorine daily (McClure, 1946). The amount absorbed through the gastro-intestinal system is too small to have any detectable effect on metabolism. Only certain teas, fish, and bone-meal preparations may contain quantities of fluorine significant in nutrition.

Water containing fluorine is the major source of this element. If water contains 1.0 p.p.m. of fluorine, approximately 1.2–1.6 mg. of fluorine is consumed daily.

Relation of Fluorides to Dental Caries in Areas Where Fluoride Occurs Naturally

The epidemiological studies of Dean (1942) and Dean et al. (1942) clearly demonstrated that children who resided, all their lives, in areas where the water contained natural traces of fluorine had approximately one-half as much dental decay as those children who resided in areas where the water is fluoride-free. The protection against dental caries due to the ingestion of fluorides extends into adult life (Russel and Elvove, 1951).

It was observed by Dean and his co-workers that among the children using fluoride-bearing water the incidence of dental caries was very low, whether or not the teeth showed signs of fluorosis. Subsequent studies showed that a fluoride concentration of 1 p.p.m. afforded maximum protection against dental caries with a minimum amount of dental fluorosis. Below this level there is little improvement in caries protection, and above 2 p.p.m. most of the children show observable fluorosis (Dean, 1942). This observation quite naturally led to the artificial fluoridation of water supplies for the partial prevention of dental caries.

Artificial Addition of Fluorides to Water Supplies

As early as 1945 certain communities—Brantford, Ontario (Brown, H. K., 1952, Hutton et al., 1951); Newburgh, New York (Ast et al., 1951); and Grand Rapids, Michigan (Cox and Ast, 1951)—adjusted the fluoride content of their water supplies to the recommended 1 p.p.m. The effect of this controlled fluoridation has been to gradually reduce the dental caries attack rate to the level observed in natural fluoride-bearing regions.

Mechanism by Which Fluorides Reduce Dental Caries

Fluorides may reduce dental caries by two possible mechanisms.

1. Fluoride in certain concentrations inhibits many bacterial enzymatic reactions. Bacteria are known to be involved in the production of dental caries. Fluoride in a concentration of 1 p.p.m. does not inhibit bacterial activity very significantly. However, during a carious attack fluoride ions may be liberated from the tooth substance in a concentration sufficient to inhibit certain bacterial activity.

2. Incorporation of fluoride into the apatite lattice structure makes the tooth more resistant to acid etching.

Either or a combination of these mechanisms may form the basis for prevention of dental caries by fluorides.

Water as a Vehicle for Fluoridation

Water was chosen as the vehicle for the addition of fluorides presumably because water is the major natural source of the element. It has other obvious advantages. It is, from a public health standpoint, a practical and easily controlled measure, requiring little if any public co-operation. Water fluoridation may be made available to large sections of the population at a very reasonable cost. The average cost per capita, for cities, is approximately ten

cents a year.   This is very favourable when we consider that the average Canadian family spends over twenty-six dollars a year for dental services. Water containing fluoride may have a beneficial topical effect while passing over the teeth (Klein, 1948). Large sections of the world population (over 3,000,000 in the United States) are already served by waters containing trace amounts of fluoride. When communal water supplies are used as a vehicle for fluoridation, the benefits of the measure will be made available to larger metropolitan areas only. It is encouraging to note, however, that at least 177 communities of less than 10,000 population in the United States are now fluoridating their water supplies.

It has been suggested that fluorides be added to milk, salt, bread, or some other vehicle rather than water. At present all these alternatives are untested. The difficulty of regulating and adequately controlling fluoridation of milk, and the limited consumption of this product as compared to water, are some obvious disadvantages of adding fluorides to milk. Ingestion of fluorides in a non-liquid media as salt will eliminate the beneficial topical effects that result from the passing of fluoride-bearing water over the teeth. It is important to note that the principle of water fluoridation does not necessarily imply that ingestion of a given quantity—for example, 1 mg. of fluoride per day—will reduce caries; rather it is based on the observation that the presence of approximately 1 p.p.m. of fluoride in water results in a substantial reduction of dental caries.   The usefulness of procedures other than incorporating fluorides in water must await the accumulation of sufficient scientific data.

Metabolism of Fluoride

A discussion of the metabolism of fluoride is essential as a background for the subsequent evaluation of the toxicity problem. Metabolism of fluoride will be discussed by referring to individual tissues or organs of the body.

1. Bones and Teeth

The fluorine content of teeth that decay appears to be lower than that of sound teeth. Armstrong (1937) reports that the mean fluorine content of sound enamel is 0.0111 per cent, while enamel from carious teeth contained only 0.0069 per cent. Teeth are highly sensitive to fluoride intake. In areas where the fluoride content of water is 1 p.p.m., a mild fluorosis, which causes no staining and is not detrimental to the appearance of the teeth may be detected in approximately 10 per cent of the children. Where the fluoride content exceeds 2 p.p.m. most children show observable signs of fluorosis of the teeth.

The bones of animals (including man) average 0.05 per cent fluorine in the inorganic material (Klement, 1933).   There is a strong possibility that a "normal" increase in the fluorine content occurs in skeletal and perhaps in dental tissues with increasing age (Glock et al., 1941). The normal fluorine content of ribs is 0.03 to 0.14 per cent. Roholm (1937) showed that the fluorine content of the ribs of two cryolite workers (ages 68 and 52) exposed to factory dust for 15 years and 8 years respectively, equalled 0.65 to 0.72 per cent. Wolff and Kerr (1938) found that the long bones of individuals with non-

disabling fluorosis contained 0.18 to 0.29 per cent of fluoride. They concluded that skeletal tolerance was of the order of 0.2 to 0.3 per cent of fluoride.

2. Saliva

Available evidence indicates that the excretion of fluorine through saliva is not of major importance. The fluorine content of saliva is in the order of 0.1 p.p.m. and is not influenced by the fluorine content of drinking water up to 1.8 p.p.m. (Cox, 1952).

3. Urine

Excretion of urinary fluorine bears a constant relation to fluorine absorption. The average urinary fluoride excretion by groups of young men closely approximates the concentrations that were found in the water they drank over the range of 0.2 to 4.7 p.p.m. McClure (1946) found that when 3.0 to 4.0 mg. of fluorine were ingested daily more than 90 per cent was eliminated by the kidney in healthy adults. The excretion also appears to be related to the amount of fluoride previously stored (Largent, 1952).

4. Sweat

The average fluorine content of undiluted sweat is 0.514 ± 0.0666 p.p.m. McClure (1946) found that in a group of five young men (19–24 years) who ingested 3.0 mg. of fluorine daily, undiluted sweat contained from 0.5 to 1.8 p.p.m. fluorine. Thus a highly significant amount of fluorine may be eliminated by the skin. This aspect of fluorine metabolism is of practical significance in areas with warm climates where large volumes of water may be consumed.

5. Blood

According to Smith et al. (1950), a 23-fold increase in fluoride content of drinking water (from 0.06 to 1.35 p.p.m.) results in a three-fold increase of blood fluorine. The maximum observed level was 0.1 p.p.m.

5. The Thyroid

It has been suggested by Wilson (1941) that the distribution of endemic goitre in Punjab and in Essex, England, is related to the geographical distributions of fluorine and mottled teeth. No reference was made to the possibility of iodine deficiency existing in these regions. The reports by Evans and Phillips (1938), Chaneles (1930), and Tolle and Maynard (1931) give no evidence that fluorine interferes with thyroid function in the concentrations recommended for water fluoridation.

Toxicity of Fluorides

The problem of acute poisoning from fluorides due to the ingestion of large single doses of fluorides is not pertinent to the present discussion. The approximate lethal dose for man is two grams (Roholm, 1937). To consume this much fluoride 2,000 litres of water would have to be ingested. A rather improbable happening! However, it is of vital and immediate concern to determine the effect of a long-term ingestion of small amounts of fluoride as contained in potable waters. Evidence on this aspect of fluoridation is available from studies of naturally and artificially fluoridated areas.

Chronic Toxicity

Numerous studies have been done in naturally fluoridated areas to determine possible detrimental effects of long-term ingestion of fluorides. Endemic skeletal fluorosis has been reported from China in malnourished persons who used water containing 5.9, 6.3 or 13.1 p.p.m. of fluoride (Lyth, 1946). Moller and Gudjonsson (1932), in an examination of 78 cryolite workers, found that 30 complained of stiffness and pain and showed x-ray evidence of osteosclerosis. It was estimated that 14 to 20.4 mg. of fluorine in dust was inhaled daily. The average deposition of fluoride was about 12 mg. per day absorbed over a period of more than 20 years. Autopsy examination of two persons who died of intercurrent disease showed osteosclerotic changes in bone, but no histological changes were observed in other organs.

Clinical examinations, experimental findings and epidemiological data indicate that only when the fluoride content of a water supply exceeds 5 to 6 p.p.m. will its prolonged usage give rise to detectable osseous changes, and then only in the most susceptible persons (Heyroth, 1952).

Thorough clinical and radiographic examinations on 1,400 high school boys and 1,700 draftees (McClure, 1944 and 1946), grouped according to fluorine exposure levels (0 to 1.8 p.p.m.) in the water supply, revealed no significant differences in the numbers of bone fractures, in the stature or weight of the individuals.

Hodges (1941), in a study of 31 persons ranging in ages from 18 to 68 years, at Bureau, Illinois, where the water contained 2.5 p.p.m. of fluorine, found no evidence of skeletal fluorosis.

Medical studies in the Newburgh-Kingston area (Schlesinger et al., 1953), where the water is artificially fluoridated, revealed no differences in the fluoride and the non-fluoride control areas. This report covered a period of six years since the introduction of fluorine into the Newburgh water supply and included complete physical examination, blood and urine analysis, roentgen studies, and special eye and ear examinations.

Studies dealing with the effect of fluoride on periodontal tissues reveal no differences between fluoride and non-fluoride areas. Elvove and Russel (1952) reported that the rate of missing teeth among natives of Colorado Springs (ages 20–44) who used water containing 2.5 p.p.m. of natural fluorides was "appreciably but not significantly" less as a result of periodontal disease than were the rates in Boulder, Colorado, where the water had less than 0.1 p.p.m.

That an ample margin of safety is afforded by the use of 1 p.p.m. of fluorine in water, is illustrated by the following calculation. The skeletal tolerance to fluoride in man is in the order of 0.25 per cent (Wolff and Kerr, 1938). The weight of bone tissue in a man weighing 65 kgs. is approximately 18 per cent (Wetzel, 1941) or 11.07 kgs. Thus 29.0 grams of fluoride would have to be stored to approach the tolerance limit. The total amount of fluoride ingested by a man consuming water containing 1 p.p.m. of fluorine for 65 years at the rate of 1.5 litres a day would be 35 grams. To obtain a level of fluoride in the bones associated with fluorosis, 83 per cent of the ingested fluoride would have to be stored. The work of McClure (1946) indicates that approximately 10 per cent of absorbed fluoride is stored and the rest is excreted. Thus storage

of fluoride even approximating the skeletal tolerance is highly improbable when water containing 1 p.p.m. of this element is consumed.

CONCLUSION

One must recognize that the ramifications of a procedure such as the addition of fluorides to water are very numerous and that the experimental investigations are never complete. There are questions regarding the mechanism of action and metabolism of fluoride that are unknown. There are similar unanswered questions regarding the metabolism of calcium, iodine, cobalt, iron, etc. However, the large number of completely negative findings regarding the toxicity of fluorides, in the recommended dosages, and the irrefutable evidence regarding its value in the partial prevention of dental caries justifies the conclusion that . . . "the evidence as a whole is consistent in offering assurance that bringing the fluoride concentration in communal water supplies to that known to be optimal for dental health is a prophylactic public health procedure that has an ample margin of safety" (Heyroth, 1952).

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FORTY-SECOND ANNUAL MEETING

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Evaluation in Health Education

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WE have often thought of coordination, motivation, and evaluation as three catchwords in health education. In previous papers, presented at meetings of the Association, we have dealt with co-ordination and motivation. We shall now attempt to clarify our thinking about evaluation.

One experience has made us very modest about this undertaking. Years ago, we were on a sub-committee of the American Public Health Association which was charged with the preparation of a report on evaluation. Some preliminary correspondence was exchanged with its chairman, but the sub-committee held no meeting and presented no report before it was dissolved.

It seems to us that much of the confusion about evaluation—and there is plenty of it—is due to the fact that we do not distinguish clearly the various elements to be evaluated. We propose to consider here three distinct aspects of evaluation in health education: materials, activities, and results.

MATERIALS

Materials can be discussed in terms of cost, quality, and effectiveness.

The *cost* of health education material is a problem similar to cost accounting in other fields. To the price of the raw material is added the cost of the various operations until it is a finished product. Cost accounting becomes very difficult when done in retrospect; it is far easier to check the various operations from the beginning, in sequence. If volunteers have given their time to some of the processes, the actual cost is not an indication of the true value of the product.

It is also possible by the same method to compute the cost of a health education service: the salaries of the workers and the other administrative expenses, such as rent, travel, etc.

The *quality* of a piece of material is more difficult to evaluate; but it can be done, although not mathematically.

Beard (3) has suggested that "evaluation, like good nutrition, should start before birth; therefore good evaluation should start from the moment a piece of material becomes an idea in some person's mind."

A committee of experts can be more useful during the preparation of any pamphlet or film than after it is completed. Such assistance should be requested as early as possible.

If a piece of material already produced is to be evaluated, a similar com-
mittee, by studying how it meets the criteria of quality applicable in the
particular case, may express an opinion regarding the scientific exactitude of
the subject matter; they may say whether the piece is for general use or only
for certain groups and under certain conditions. They may suggest the
quantity to print or purchase. They may propose to revise a text or even to
discard it completely. The committee takes for granted, or ascertains, as the
case may be, that the piece of material will meet a definite need and will be
be used with proper motivation. In the first place, they have taken into
account the reputation of the author as an expert, his professional
prestige, and his other qualifications.

The *effectiveness* of materials is different from their quality, although the
latter is a requisite of the former. Materials of quality may be ineffective if
not used properly, either by the distributor or the consumer.

It would seem that a good way to assure effectiveness is to subject a new
piece of material to some "pretesting", as proposed by Knutson (4). Materials
in rough form can be offered to some of the group for whom they are intended
and these persons can be asked to point out any parts they think some people
might not understand. This procedure will identify causes of confusion and
suggest methods of improvement.

Evaluating the effectiveness of the final product is equivalent to assessing
the results of health education. This point will be discussed later.

ACTIVITIES

Evaluating a program and its component activities is a different proposition.
Again we must see clearly the elements to be evaluated. We shall leave aside
the quantity of health education and limit ourselves to the more efficient
aspect of quality. Quality may be assessed under the headings of coverage,
subject matter, method, and cooperation.

Coverage

The load of the health educator should be estimated in order to appreciate
the distribution of the service among the population and its communities.
Which groups have been approached? To what extent? In which communities?
Were they preexisting groups or are they the result of community organization
for health education? Was the program reasonably continuous?

Subject Matter

What subjects have been treated? Were they chosen by the groups them-
selves? Did they correspond to general needs or specific problems that edu-
cation can help to solve? Do they comprise the most important aspects of
personal and public health that should concern adults in any community?
Does the health educator take advantage of the educational opportunities of
routine or special activities? Are the campaigns or drives launched by outside
agencies well integrated in the local program?

Method

Are the various communication media being utilized in a coordinated
fashion? Or is the use of any one neglected? Does the health educator exploit

the motives that may lead to action, according to the needs and interests of the people?

Cooperation

Does the health educator work in cooperation with the other members of the staff and vice versa? With the health officer? With the province or state division of health education? (Although it is very difficult to carry out an active program of health education without a person specially responsible for it, yet the program must not be a restricted one, reserved to the health educator; far from it.) Is the program well coordinated with those of the voluntary agencies and the school teachers?

We have checked these points of methodology in the many field visits we have made to public health educators. The answers we received have given us a good overall picture of the local program, with its strong and weak points, and have made it possible to give many pertinent suggestions.

RESULTS

Finally, to measure the results of health education is by far the most difficult problem in evaluation. As Beard, our former Canadian colleague, has so aptly said: "A thorough and complete evaluation of the influence of health education literature on those who read it would require extensive surveys over a period of years, and a great variety of tests to measure the changes in health practices, to say nothing of the exhaustive research necessary to eliminate the influence of other factors."

The method used in a recent research project has been described in the September issue of the American Journal of Public Health (5). The reader is astounded by the infinite care and the numerous precautions taken by the authors in elaborating their technique. Surely this is likely to discourage anyone from attempting to evaluate the results of health education.

Furthermore, their last paragraph reads as follows: "This evaluation shows that in order to affect attitudes and practices, more must be known about how to utilize the complex psychological factors which stimulate persons to action. Also, the alteration of established patterns of behavior may be too much to expect from a single instrument."

Let us hope that research will find simple techniques that will separate and measure accurately the results of education achieved by each of several persons, when using each of several media; that will recognize among the results those that belong to official organizations, the ones to be credited to voluntary agencies, even the results of education from person to person; and possibly the training value of good example.

To be really effective, the simple technique will have to dissociate the complex influence of outside factors from that no less complex source of education itself. We once gave a lecture on tobacco, in a series on personal hygiene, to a class of medical students. The week after, we asked the students if any of them had reduced their consumption of tobacco; a few raised their hands. We were making a comment of satisfaction about this tangible result of health education, when one of the group recalled that the price of cigarettes had gone up during the week.

Frankly, until research has progressed materially toward the correct evaluation of the results of health education, we might listen to the advice given by Beard when she wrote: "Evaluation is not a process which can be applied now and then, once a month, or once a year, like an auditor's report. It must be a continuing process, day in and day out—a habit or a state of mind in which we constantly utilize opportunities to assess the usefulness of the materials distributed, and where possible to increase that usefulness."

One thing is certain, and it has been emphasized by the Public Health Education Section of the American Public Health Association in a news letter dated June 1952: "We need to test the materials we distribute in order to check our evaluations. In the final analysis this is the only way that we can learn to make better judgments. For most of us this cannot be done by detailed research techniques but must be done on the run. In doing this we may get better results by a rambling informal interview asking indirect questions which do not force the person interviewed to admit that she didn't understand the pamphlet, movie or whatever we are inquiring about."

Even though we cannot yet measure accurately the results of health education, we are firmly convinced of its importance and its true usefulness.

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Tissue Culture Methods in the Laboratory Diagnosis of Cases of Poliomyelitis*

WITH OBSERVATIONS ON THE BEHAVIOUR OF COXSACKIE VIRUSES IN TISSUE CULTURE

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FOLLOWING the observations of Enders, Weller and Robbins (3) that poliomyelitis viruses can be cultivated in tissue culture, it has been shown that this technique can be used in the isolation of strains from pathological specimens, especially stools, and in typing (8, 9, 10, 13, 17, 18). Hope has been expressed that the technique might replace the use of monkeys for the isolation of virus.

In Toronto, several studies on the growth of poliomyelitis virus in tissue culture have been carried out (1), leading to the development of methods of producing virus in large quantities (4). We have also isolated and typed strains of virus responsible for an outbreak of poliomyelitis in the Northwest Territories in 1949 (16), and in other parts of Canada.

During 1952, tissue culture methods were used in the study of cases of suspected poliomyelitis admitted to The Hospital for Sick Children, Toronto. These tests were carried out in the hospital Virus Research Department to determine whether such procedures might become routine. Monkeys were also inoculated to compare the sensitivities of the two methods. Since many patients suffering from clinical poliomyelitis excrete Coxsackie and poliomyelitis viruses, tests for the presence of Coxsackie virus were also performed.

CASES STUDIED

The 1952 outbreak of poliomyelitis in the City of Toronto was unusual, in that of 182 reported cases, 136 were non-paralytic; the ratio of paralytic to non-paralytic cases was thus 1:3. The picture in our hospital was similar. Of 93 patients admitted between July 1 and October 20, 73 were non-paralytic. Of these 93 patients, 33 non-paralytics and 10 paralytics were tested for the

presence of Coxsackie and poliomyelitis viruses in stools. Serological tests were performed also, but will be reported later.

METHODS USED IN CLINICAL STUDY

All patients studied were closely examined by a full-time research fellow, at or shortly after admission, daily during their hospital stay, and at a follow-up clinic after discharge. Specimens of blood, cerebrospinal fluid, and stools were taken for various tests, including virus studies.

METHODS USED IN VIRUS STUDY

Preparation of Stools for Inoculation

Method A. A 10% suspension of stool in saline was filtered through sterile gauze, then centrifuged in a "Spinco" high-speed centrifuge at 10,000 r.p.m. for 30 minutes at 4° C. The supernatant, treated with 1,000 units of penicillin and 1,000 micrograms of streptomycin per ml., is referred to as the "moderate-speed supernatant".

Method B. A 10% suspension of stool in saline was filtered through sterile gauze, then clarified by centrifugation in the "Spinco" machine at 4,000 r.p.m. for 20 minutes. The supernatant was then centrifuged at 40,000 r.p.m. for one hour at 4° C. The supernatant was discarded, and the deposit resuspended in about 2 ml. of sterile saline. This suspension was then shaken twice with a double volume of washed anaesthetic ether, which was removed under vacuum. This suspension (with antibiotics) is referred to as the "ultracentrifuged deposit".

Inoculation of Monkeys

A quantity of 0.8 ml. of resuspended ultracentrifuged deposit of stool was inoculated into both thalami of a rhesus monkey. Monkeys were examined daily and were killed on the first day of paralysis. Animals showing no paralysis were killed four weeks after inoculation. Portions of cerebral cortex, thalamus, brain stem, and spinal cord were placed in 10% formol-saline and examined histologically. Portions of the spinal cord enlargements of paralyzed monkeys were stored frozen.

Inoculation and Examination of Suckling Mice

Two-day-old suckling mice were randomized and distributed eight or nine to a mother; 0.03 ml. of the moderate-speed supernatant preparation was inoculated intracerebrally and 0.1 ml. peritoneally. All mice were examined daily for signs of illness, and were killed as soon as any were noted.

Mice to be examined histologically were placed in 10% formol-saline. Penetration of the fixative was ensured by a ventral incision into the abdominal cavity and a dorsal incision into the skull cavity. After fixation for several days, the musculature of two limbs, two levels of the brain, and three levels of the trunk were sectioned. Muscle, brain and cord, dorsal fat pads, lungs, heart, liver, and pancreas were examined for the presence of lesions typical of Coxsackie virus infection.

Inoculation of Tissue Cultures

Flask Technique

Medium. Synthetic Mixture No. 199 (6), sterilized by Seitz filtration under pressure, was used with the addition of 100 units of penicillin and 100 micrograms of streptomycin per ml.

Tissue. Testes from rhesus monkeys were removed aseptically from the tunica and washed several times in Mixture No. 199. Testes were then minced until the fragments measured about 1.5 mm.; these were washed twice.

Preparation of Cultures. Two drops of a concentrated suspension of minced tissue were added from a Pasteur pipette to 3 ml. of Mixture No. 199 in a 25 ml. Erlenmeyer flask. The flasks were stoppered with white rubber stoppers and incubated at 37° C. overnight. The following day, the nutrient fluid was replaced with fresh.

Inoculation of Cultures. A quantity of 0.1 ml. of reconstituted ultracentrifuged deposit of stool was added to each of two cultures.

Control Cultures. In each group, six flasks of uninoculated tissue suspension served as controls.

Fluid Changes. The supernatant fluids of the cultures were replaced twice weekly for four weeks.

pH. The pH of the culture fluids was read by visual comparison with pH standards in similar flasks. The readings were made by the same worker under similar conditions before each change of fluid.

Roller Tube Culture Technique

Media. Two nutrient media were employed:

(1) *"Natural feeding mixture"* was used in the earlier stages of the work to initiate outgrowth of cells in roller tube cultures. This was a mixture of 3 parts Hanks' balanced salt solution and 1 part Simms' ox-serum ultrafiltrate (3), with the addition of 2.5% beef embryo extract, 10% normal horse serum, and antibiotics.

The beef embryo extract was prepared according to the method of Enders (2), with minor modifications.

The horse serum was obtained from a horse proven to yield a satisfactory serum.

(2) *Mixture No. 199*, employed more recently, has proven highly satisfactory, and is now used in place of the natural feeding mixture in all phases of tissue culture work in our laboratory.

Tissue. Rhesus monkey testes were minced until the fragments measured less than 1 mm.

Preparation of Cultures. One drop of reconstituted chick plasma (Difco) was introduced into a chilled 15 × 150 mm. pyrex test tube and spread evenly over the lower third. Six fragments of tissue were then embedded on the plasma-lined area. One drop of reconstituted chick embryo extract (Difco) was added, and the tube rotated until the plasma clotted. Two ml. of medium were added, and the tubes were rotated (12 revolutions per hour) at 37° C. for

six days before infection. When the natural feeding mixture was employed, the pH fell below 7.0 in approximately three days, necessitating replacement. With the more effectively buffered Mixture No. 199, this change was not necessary.

Inoculation of Cultures with Virus. After incubation for six days, cultures showing a satisfactory outgrowth of fibroblasts were selected. The medium was removed, and 0.2 ml. of inoculum plus 1.8 ml. of Mixture No. 199 was added. The tubes were then rotated and incubated as before.

Cytopathogenic Changes. In the presence of poliomyelitis virus, the fibroblasts became granular and finally disintegrated completely. This "cytopathogenic effect" was visible under the low power of the microscope three to seven days after inoculation.

Titration of Viruses. Serial tenfold dilutions of the fluid to be tested were made in sterile normal saline with a separate pipette for each dilution. Dilutions were inoculated (0.2 ml.) into groups of five roller tube cultures and 1.8 ml. of Mixture No. 199 were added. Six control tubes received 2 ml. of medium only. The tubes were rotated at 37° C., and examined daily for cytopathogenic changes. The medium was not changed during the seven-day observation period. Endpoints were generally very sharp, and titres were expressed as 50% cytopathogenic doses (CPD_{50}), calculated by the Kärber method.

Isolation of Poliomyelitis Virus from Stools

Reconstituted ultracentrifuged deposit of stool (0.1 ml.) was inoculated into each of two flask cultures of monkey testis. Cultures were incubated for four weeks and changes of fluid made twice weekly. The presence of poliomyelitis virus was suggested if the pH did not fall in the later stages of the culture. Fluids removed from the flasks at changes 2, 4, 6, 7, and 8 were stored frozen. Fluid changes 4, and 7 or 8 were later inoculated into suckling mice to test for Coxsackie virus, and into roller tube cultures to detect poliomyelitis virus. These roller cultures were examined daily for cytopathogenic changes, and the medium was removed and replaced on the seventh day. If the fibroblasts remained normal for two weeks, it was concluded that no poliomyelitis virus was present in the inoculum. If cytopathogenic changes occurred, fluid was subinoculated into groups of three roller tube cultures, which were examined daily for evidence of necrosis. The culture fluids were replaced twice weekly for two weeks, and fluid changes 2, 3, and 4 were pooled and stored frozen for typing at a later date.

Typing of Suspected Poliomyelitis Strains

A volume of undiluted tissue culture virus pool was mixed with an equal volume of each of the following: (a) normal rhesus monkey serum, diluted 1: 2, (b) Type 1 monkey poliomyelitis antiserum (Brunhilde), diluted 1: 2, (c) Type 2 antiserum (Lansing), diluted 1: 2; (d) Type 3 antiserum (Leon), diluted 1: 2. These mixtures were left at room temperature for 1½ hours, and 0.2 ml. volumes were then inoculated into groups of five roller tube cultures. The final readings were made on the seventh day after inoculation. The type of

the virus strain being studied was indicated by inhibition of the cytopathogenic effect in the presence of one of the three type-specific sera.

Some of the agents producing cytopathogenic changes were not inhibited by the three sera. These agents were subcultured in roller tube cultures and preparations were then inoculated in monkeys and suckling mice, and a titration was performed by the inoculation of tenfold dilutions of tissue culture fluid from $10^{-3.0}$ to $10^{-6.0}$ into groups of five roller tubes.

Tests for Coxsackie Virus

Stool extracts (Method A) were inoculated into suckling mice; animals becoming sick were killed and examined histologically. The methods were similar to those described previously (7, 11). Strains were classified into Group A or Group B on the basis of the histological changes in suckling mice.

RESULTS

Clinical Study

The non-paralytic patients were typical cases of virus meningitis with regard to clinical features and changes in the cerebrospinal fluid. In the summer months, there was every justification for regarding these patients as suffering from non-paralytic poliomyelitis. The paralytics were likewise quite typical, and there was no reason to question the accuracy of the diagnosis.

Virus Study

The stools of 33 non-paralytics and 10 paralytics were tested for Coxsackie and poliomyelitis virus by the inoculation of suckling mice, monkeys, and flask tissue cultures with stool. The results are summarized in Table I.

From Table I it will be seen that 18/33 non-paralytics excreted Coxsackie virus. Details of the classification of these strains are given in Table II. Six patients excreted poliomyelitis virus; three of these strains were recovered only in monkeys, two both in monkeys and tissue culture, and one in tissue culture only. The three strains isolated in tissue culture belonged to Type 1. Seven agents produced cytopathogenic changes in tissue cultures but were not neutralized by poliomyelitis anti-sera. These agents were further studied (see below). Five patients excreted both poliomyelitis and Coxsackie virus.

With regard to the paralytics studied, only one excreted Coxsackie virus. Poliomyelitis virus was recovered from nine of the ten patients; of the six strains recovered in tissue culture, four belonged to Type 1 and two to Type 3. Only a single unidentified agent was isolated. This agent was investigated together with the seven recovered from non-paralytics. One patient excreted both Coxsackie and poliomyelitis virus.

In all, eight agents resembling poliomyelitis virus with respect to the production of cytopathogenic changes were isolated. It was thought that in some instances the titre of these agents might have been unusually high, and therefore a titration of each was performed. Each strain was sub-cultured twice more, and the fluids from the fourth sub-cultures were employed as virus pools, which were titrated in groups of five roller tube cultures. The titres of three strains, 5, 8, 16, were sufficiently high (over $10^{-4.0}$) to warrant repetition of

TABLE I

POLIOMYELITIS, TORONTO, 1952: RESULTS OF TESTS FOR PRESENCE OF COXSACKIE
AND POLIOMYELITIS VIRUSES IN STOOLS OF 33 NON-PARALYTIC AND
10 PARALYTIC PATIENTS

Type of illness	Reference number of patient	Virus isolated from stool			Unidentified agent present in tissue culture
		Coxsackie virus isolated in suckling mice	Poliomyelitis virus isolated in		
			monkeys	tissue culture (type as specified)	
Non-Paralytic Poliomyelitis	6, 7, 11, 12, 14, 17, 22, 28, 31, 32.	+	−	−	−
	13, 20	+	+	−	−
	8	+	+	−	+
	18, 24	+	+	Type 1	−
	21	+	−	Type 1	−
	15, 16	+	−	−	+
	5, 10, 26, 30	−	−	−	+
	1, 2, 3, 4, 9, 19, 23, 25, 27, 29, 33.	−	−	−	−
	Total Isolations	18	6	3	7
Paralytic Poliomyelitis	60	+	+	−	−
	55, 57	−	+	Type 1	
	62	−	not tested	Type 1	
	58	−	+	Type 3	
	59	−	not tested	Type 3	
	63	−	+	−	+
	54	−	+	−	−
	61	−	not tested	−	−
	56	−	−	Type 1	−
	Total Isolations	1	6	6	1

TABLE II

POLIOMYELITIS, TORONTO, 1952: ISOLATION OF COXSACKIE VIRUSES FROM
STOOLS IN MICE AND IN TISSUE CULTURES

Reference number of patient	Type of illness	Result of inoculating stool into suckling mice:		Result of inoculating into suckling mice fluid changes from flask tissue cultures inoculated with stool:			
				Fourth fluid change		Eighth fluid change	
		Group A virus isolated	Group B virus isolated	Group A virus isolated	Group B virus isolated	Group A virus isolated	Group B virus isolated
60	Paralytic	+	−	−	−	−	−
15, 16, 20, 28,	Non-paralytic	+	−	−	−	−	−
7, 11, 17, 22,	Non-paralytic	−	+	−	+	−	+
6, 14, 18,	Non-paralytic	−	+	−	+	−	−
12, 31,	Non-paralytic	−	+	−	−	−	+
8, 13, 21, 24, 32.	Non-paralytic	−	+	−	−	−	−

the typing test, a fixed dose of 100 CPD_{50} of the agent being used. The results of these repeat tests were, however, identical with the first.

In order to investigate these strains further, material from the fourth sub-culture was inoculated into suckling mice, and in the case of six of the agents, also into monkeys. None of the litters of suckling mice showed any clinical or histological evidence of Coxsackie infection. None of the monkeys showed clinical or histological evidence of poliomyelitis.

It is planned to test several of these unidentified agents with acute and convalescent phase serum from the patients concerned. To date, such a test has been carried out in the case of patient no. 8 (Table I). The acute phase serum was taken 3 days and the convalescent 5 weeks after onset. The 50% neutralizing titre of the acute phase serum tested with 100 CPD_{50} of the agent was $< 10^{-1 \cdot 0}$, and of the convalescent phase serum was $10^{-2 \cdot 3}$. These results suggest that the unidentified agent proliferated sufficiently actively to stimulate the development of specific antibody.

Eighteen strains of Coxsackie virus were recovered from non-paralytics and one strain from a paralytic. Five of the strains belonged to Group A and fourteen to Group B. The strain isolated from the paralytic case was a Group A. The stools containing these strains were all inoculated in flask tissue cultures, the fourth and eighth fluid changes from which were inoculated into suckling mice. The results of these tests are presented in Table II. None of the Group A strains proliferated in tissue culture. Five of the Group B strains likewise failed to grow in tissue culture. Six of the Group B strains appeared to proliferate, as the virus was recovered from the eighth fluid change. Three strains were recovered from the fourth fluid change but not from the eighth, so presumably did not proliferate.

DISCUSSION

Our experience has shown that the tissue culture technique can be used routinely in a hospital virus laboratory for the isolation of poliomyelitis virus. However, the particular type of technique that we have used, in which stool extract is placed in flask cultures of monkey testis before sub-culturing into roller tubes, does not appear to be as sensitive to the presence of poliomyelitis virus as does monkey inoculation. Of thirteen stools which were tested both in monkeys and in tissue cultures, six isolations were made only in monkeys, tissue cultures proving negative; five strains were recovered both in monkeys and tissue cultures; two strains were isolated in tissue cultures only.

In previous experiments with strains of virus well-adapted to growth in tissue culture, we have found that monkey kidney is a preferable source of tissue to monkey testis, and allows of considerably greater virus production (1). Currently, we are employing flask cultures of monkey kidney in diagnostic tests, in the hope that such cultures may prove somewhat more sensitive, thus increasing the usefulness of the tissue culture method. However, it must be stressed that, in our observations at least, the tissue culture technique presently employed cannot adequately replace monkey inoculation.

Once again we have demonstrated the frequency with which Coxsackie virus can be found in the stools of poliomyelitis patients. In this series, of

33 non-paralytics, 18 excreted Coxsackie virus; of 10 paralytics, only 1 excreted this virus. The strains were predominantly of the B Group. The presence of Coxsackie virus does not seem to interfere with the proliferation of polio-myelitis virus in tissue culture. The Coxsackie strains did not produce cyto-pathogenic changes in roller tube cultures, nor could they be recovered after passage in such cultures, although a few strains appeared to proliferate in flask cultures.

In addition, eight unidentified agents, including one from a paralytic patient, were isolated in tissue cultures. Similar agents have been reported by others (5, 8, 13, 14). These agents produced typical cytopathogenic changes in tissue cultures, but were not neutralized by any of the three type-specific sera. They were not pathogenic for monkeys or suckling mice. The two-phase sera of one patient were tested for homologous neutralizing antibody. A marked rise in titre occurred. The significance of these agents in the overall picture of poliomyelitis cannot be fairly evaluated at the moment, and intensive work is necessary. It is of interest to note that Steigman has recovered a similar type of agent from a human spinal cord (12).

We have now typed, by tissue culture methods, twenty-three strains of poliomyelitis virus isolated in Canada from 1948 to 1952 inclusive. Twenty Type 1, no Type 2, and three Type 3 strains have been identified. Analysis of a much larger number of strains isolated from many parts of Canada in 1953 is now in progress and will be reported shortly.

SUMMARY

1. An attempt was made to isolate poliomyelitis viruses from the stools of 33 non-paralytic and 10 paralytic cases of poliomyelitis admitted to The Hospital for Sick Children, Toronto, during the summer of 1952, using the technique of tissue culture.

2. Tissue cultures of monkey testicular tissue were used to detect the presence of poliomyelitis virus in pathological specimens; monkeys were also inoculated cerebrally.

3. Suckling mice were injected to detect Coxsackie virus.

4. Poliomyelitis virus was isolated from the stools of 6 out of 33 non-paralytic and 9 out of 10 paralytic cases.

5. Six strains were isolated in monkeys only, five in monkeys as well as tissue cultures, and two in tissue cultures only.

6. Typing of nine strains was carried out by the tissue culture technique. Seven Type 1 strains and two Type 3 strains were identified.

7. Eight agents resembling poliomyelitis viruses in their effects on tissue cultures were not neutralized by the three type specific sera; these agents remain unidentified.

8. Eighteen out of 33 non-paralytic and one out of 10 paralytic cases excreted Coxsackie viruses.

9. The strains of Coxsackie virus were classified into Group A (5 strains) and Group B (14 strains) on the basis of the histological changes in suckling mice.

10. The Group A strains and eight of the Group B strains failed to proliferate

in flask tissue cultures. Six of the Group B strains appeared to multiply in flask tissue cultures, as the virus could be recovered after cultivation for four weeks. These Coxsackie strains did not produce cytopathogenic changes in roller tube cultures, nor did they interfere with the isolation of poliomyelitis viruses from the same stools. No Coxsackie viruses could be recovered after passage in roller tube cultures.

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Public Health Statistics in Air Pollution Studies

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IN the atmosphere of most large communities there are pollutants of various types and concentrations. These are mainly products of incomplete combustion processes. The sources include stack wastes from industrial plants, domestic fuel wastes from house chimneys, smoke from vessels and trains, and exhaust fumes from cars, trucks and buses. The pollutants consist of particulate and liquid matter as well as vapors and gases.

It is well established that atmospheric contaminants in relatively high concentrations may have deleterious effects on health, producing acute or chronic illness, or even death. Industrial health experts have thoroughly documented the toxic effects of many substances used in, or products of, industrial processes. Fortunately, the concentrations in the atmosphere are, due to dispersion, far below the maximum allowable concentrations; but adverse meteorological conditions sometimes produce abnormal concentrations of the pollutants regularly present.

In the Meuse Valley, Belgium, in 1930, at Donora, Pennsylvania, in 1948, and last winter in London, England, we have had examples of sickness and death resulting from such conditions. A review of the toxicologic aspects of air pollution has been given recently by Dr. Stokinger (1).

Questions of public health significance are:

How common are these acute episodes of high concentrations and at what pollution levels is health affected?

What are the effects on health of lower concentrations of air contaminants "normally" present over long periods?

Adequate information respecting the acute and chronic effects of air pollution on health is essential to the planning and enforcement of appropriate preventive and control measures. The problem for the biostatistician, then, is to obtain for this purpose health indices which may be related to both air pollution and meteorological measurements.

There are two ways of considering the relevant health data:

(1) We can compare the health indices for a community as a whole over a considerable period with some other community which is known to be rela-

tively free of pollution. These comparisons will be subject to the difficulty that a multiplicity of factors affect health; pollution may be only a minor one and its effects not distinguishable.

(2) It is not feasible to measure the health of a population under controlled conditions of pollution, but we can compare health indices for similar population groups which are living in areas of differing average pollution level.

It is obviously practical to confine the study to one community so that some of the variables will be automatically taken care of. In addition we must compare only matched population groups in the community. Differences in health indices may then, in the absence of alternative hypotheses, be attributed to pollution levels. Studies in other communities, if conducted with the same general methodology, will, of course, increase the scope of applicability of common conclusions.

In the current Windsor-Detroit health study, which is being conducted as part of the International Joint Commission Detroit River Air Pollution Investigation, we have established areas of high and low pollution and will compare the health status of matched samples of populations in these areas. We are also using a third control area, outside Windsor but nearby, where there is little or no industrial pollution. A review of the methodology of the study, the first of its kind, has been published elsewhere (2).

What Health Indices Will Be Useful?

We are inclined to look first at mortality statistics and will be especially concerned with total mortality, the crude death rate from all causes; and specific mortality rates, including infant mortality, a recognized index of the health of a community, and rates for cardio-vascular and respiratory diseases.

These statistics should be reviewed for a considerable period of years so that the trends of levels in the community may be compared with those elsewhere. More directly, we should compare the mortality statistics for high and low pollution areas in the same community. In the present study we are able, with the co-operation of the Dominion Bureau of Statistics, to follow both procedures. For the second we are assigning deaths to census tracts and grouping these to correspond to high and low pollution areas.

Morbidity Statistics

But mortality statistics are at best an indirect and incomplete measure of the health of a community. They are concerned with the zero end only of the spectrum of health status.

Morbidity measurements cover the whole range of ill health. These should therefore be more useful to us.

Sources of morbidity data available to the medical officer of health of a community may include the following:

Communicable disease cases and deaths reported.

Hospital records.

School health records.

Health clinic data (and occasionally out-patient department statistics.)

Medical care plan records.

Can we make use of these in an air pollution investigation?

Communicable Disease Reports

Apart from well-known deficiencies in reporting, these are apt to have little relevance. It might be useful, however, to examine the statistics respecting the common communicable diseases of childhood in view of the relative certainty of diagnosis and considerable volume of cases. Since we would not expect the incidence of these diseases to be associated with air pollution, they might serve to some extent as an independent basis for comparison of differences in sickness incidence and prevalence between communities. Unless the records would permit of allocation of the cases to areas of high or low pollution or to census tracts, little more could be done with them.

Hospital Records

Here the problem is the general one encountered in using hospital statistics for morbidity studies, viz., the difficulty of computing rates when we do not know the parent population. Also, hospital patients are generally selected, not representative populations. We might find it worthwhile, however, to record admissions on a current basis, along with pollution measurements, to see whether acute pollution episodes result in peak admission loads.

School Health Records

Data from school records should be more directly applicable since in general the population of a particular public school comes from the adjacent area and will be a relatively homogeneous group. In any case, it should be useful to compare the overall sickness absenteeism in an industrial area such as Windsor with the rate for Canada and for other cities. The Canadian Education Association study conducted by Dr. Phillips will provide useful comparable data (3).

More particularly, we would be interested in comparing the sickness absenteeism rates, total, and specific for respiratory diseases for example, for schools serving areas of high and low pollution.

Aside from absenteeism, we might find it of value to examine the school clinic records to determine whether any unusual incidence of complaints, such as eye irritation or coughing, coincides with times of high pollution levels. If there are peaks, do they occur in all schools or just those in high pollution areas?

Health Clinic Data

We cannot expect data from health clinics to be of much help. They seem commonly to give "work load" rather than case record information. Also, they usually do not cover large segments of the population and are mostly serving specific categories of individuals or cases of particular diseases.

Medical-care Plan Records

Because of the increasing extent to which people are obtaining medical care under pre-payment plans of various kinds, we have an additional and more direct source of morbidity data. We would hope that this would tell us the volume and types of illnesses and the number and characteristics of the individuals affected. However, the records are not always susceptible of such analysis when they are maintained primarily for financial purposes.

These basic sources of public health statistics can at best provide only

supporting evidence, and will not by themselves be adequate for our study. They are obtained routinely for other purposes and are limited in their application to our problem.

Special Morbidity Study

We still need a study specially designed to measure health and pollution levels simultaneously in the same community for population groups one of which is exposed to relatively high and another to relatively low average levels of pollution.

In our Windsor-Detroit investigation, for example, we planned and are presently carrying out a pilot morbidity study of families in high and low pollution areas and in control areas outside but not far from Windsor. A corresponding investigation is being conducted in Detroit and we hope to be able to pool our results. We are obtaining sickness information by a method similar to that used for the Canadian Sickness Survey.

In Windsor and in the control areas, trained enumerators are obtaining a complete record of the sickness experience of the members of households visited every two weeks. The households in these areas are random samples matched for certain socio-economic factors believed to be correlated with health status. In addition to house assessment value, family composition, age and sex are among the factors being considered. The stratified matching process for selecting the samples has been carried out by Mr. T. G. Donnelly of the Dominion Bureau of Statistics.

Concurrently with the health measurements, the environmental chemists will be obtaining data respecting the types and levels of pollution. The meteorologists will provide information respecting humidity, temperature and air movements.

Health Indices

What precisely do we mean by "health measurements"? First of all, we are concerned with the total volume of sickness, both the number of individuals sick and the number of sicknesses during the year. We are interested, too, in any unusual distribution in the types of sickness experienced by these population groups. We shall also be looking at the incidence and prevalence of those diseases which may be indicative of, or associated with pollution. Further, we shall note the occurrence of symptoms and syndromes, because these may appear without sickness in the usual sense and have been observed in acute pollution episodes. Symptoms may also help us to arrive at more accurate diagnoses of reported sicknesses.

For purposes of an air pollution health study, sickness should be broadly defined, as in the Canadian Sickness Survey for example, to indicate "any condition a person is aware of as disturbing his state of health."

The following attributes of a sickness should be part of the record:

(*a*) Duration—period in days from "date sickness began" to "date sickness ended."

(*b*) Severity—as indicated by medical attention received; specifically, hospitalization, doctors' visits, nursing service, clinic visits.

(c) Disability—as measured by (i) days away from work or usual occupation; (ii) days in bed.

From these data, incidence, prevalence and disability rates may be computed to serve as the health indices for the population groups studied.

It will be desirable also, if there are sufficient data, to prepare the corresponding frequency distributions: the distribution of persons by number of times sick during the year, and of both persons and cases by number of days of sickness during the year.

If a high average level of pollution has a long-term effect on the health of the people, this may be reflected in differences in morbidity rates for the high and low pollution areas. Indices based on a year's experience may be adequate for valid comparison of the population groups, but the study might have to be carried on for several years to get definitive results.

In Windsor we plan also to make a simple current comparison of the sickness and pollution levels by plotting these periodically on the same graph. This should enable us to spot concurrent peaks of pollution and sickness or symptoms. Thus we may obtain, for the first time, I believe, systematic current, rather than retrospective, evidence of this relationship.

If the matching of the population groups is adequate, it will permit direct comparison of the sickness indices without an adjustment for age, sex, or other characteristics. Our data will be meagre for comparison of specific rates in any case. However, we shall still be careful to watch for evidence of any selective effect of pollution comparable to that observed in smog disasters.

Initial Health Status

Yet another measurement was suggested and adopted for our study, as it serves several purposes. We decided that we should attempt to assess the health status of the people at the time the study started. Long exposures to high pollution might result in differences in health status evident in an initial one-time assessment. Such an evaluation would also give us a base-line for consideration of subsequent illness experience. It would be desirable to have clinical examinations or at least some form of multiphasic screening, but neither was practical in our investigation at this time. As an alternative expedient, we adapted the Cornell Medical Index (4), a diagnostic screening device consisting of a simple health questionnaire. This is in addition to a record of pre-existing conditions.

SUMMARY

1. Evidence already available establishes a positive relationship between pollution and ill health under adverse meteorological conditions. The same pollutants continually present in the atmosphere of our large cities may have long-term deleterious effects. Intensive study is necessary to obtain the data needed for planning preventive and control procedures with respect to potential hazards both acute and chronic.

2. The scientific investigation of the effects on health of air pollution in concentrations normally present in industrial communities requires careful planning in view of the technical complexity of pollution and health measurements and because of the multiplicity of factors operating simultaneously.

3. The usual public health statistics should be considered, but are apt to be of limited usefulness.

4. A controlled experiment in the sense of assigning people at random to high and low pollution areas is not feasible. The nearest approach is the study of population groups in the same community matched as to socio-economic factors but exposed ·to differing average pollution levels. A study group located in an area free of industrial pollution is also desirable for comparison. Another control or comparison device is the pre-survey evaluation of health status, clinically if possible, but a health questionnaire may serve as a useful expedient.

5. We can anticipate that a carefully planned investigation will produce significant data respecting acute episodes. The chronic effects of normal pollution present a more complex problem which may require years of study for valid assessment.

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The Investigation of Soil for Bacteriophages Against Pathogenic and Saprophytic Acid-Fast Micro-Organisms*

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SOIL as a source of bacteriophages for acid-fast bacteria has been reported by several authors (1, 2, 3, 4, 5). The success of isolation of phages from soil depends primarily on the technique followed and on the nature of the soil. Soil enriched with a particular acid-fast micro-organism over a period of time has been found superior for phage isolation to unenriched soil (1, 2, 4, 6).

This paper describes briefly the investigation of soil as a source of phage for pathogenic and saprophytic acid-fast micro-organisms.

MATERIALS AND METHODS

Organisms used consisted of three strains of BCG, 141–34b, 137–17, 152–80a; two strains of *M. tuberculosis* var. *bovis*, 103, 111, supplied by Dr. Mitchell, Hull; two strains of *M. tuberculosis* var. *hominis*, 527, 580, obtained from the Provincial Laboratory, Edmonton; *M. ranae* 503, *M. eos* 500, and *M. phlei* 502, all received from the National Research Council, Ottawa. *M. eos* and *M. phlei* are original cultures, obtained from the Microbiological Institute at Göttingen, Germany, and isolated from soil by Dr. O. von Plotho. *M. ranae* was isolated from frog by Moeller and is ATCC no. 110.

The media used were TB broth base (Bacto) supplemented with 5% glycerol and sheep serum, and nutrient broth (4) containing 5% sheep serum. The solidified media contained 1.5% Bacto agar. The tubercle bacilli were grown in TB broth while the saprophytic strains were grown in nutrient broth at 37° C.

Procedure for Isolation of Phage

Two types of soil were used: dung soil (i.e., soil near a manure pile) and garden soil. Four samples of each soil (75 gm.) were used for each micro-organism. Two samples of each soil were treated every five days with 10 ml. of broth-culture growth which was centrifuged and washed in normal saline three times, and the remaining two with 10 ml. growths in broth. The inocula were so adjusted that approximately 8×10^8 cells/ml. were used. About 10–12 day old tubercle bacilli cultures and 4–5 day old saprophytic cultures were used. Enrichment of soils with tubercle bacilli cultures was carried out for 8 months. With the saprophytic strains it was found sufficient to carry out the enrichment from 1–3 months. All incubation was carried out at 37° C.

Soil filtrates, both centrifugate and seitzed, were examined for the presence of phage each month by the usual techniques (4). Controls consisted of 12 samples of soil, 6 of which were dung soil and 6 garden soil. Two samples of each soil were inoculated with Dubos medium (modified), 2 with nutrient broth, and 2 with sterile saline. For comparison 48-hour soil filtrates were examined against various strains for lytic activity.

RESULTS

Over a period of 8 months, filtrates from soils inoculated with tubercle bacilli were tested each month on homologous cultures. In no instance was there any evidence of lytic activity. The final seitzed filtrates were tested against 15 saprophytic and 20 human tubercle bacilli. There was no indication of phage activity.

Two samples of dung soil inoculated with a heavy washed suspension of *M. ranae* yielded a bacteriophage for that strain after 6 weeks of enrichment. A bacteriophage for *M. eos* was isolated from one sample of dung soil after 12 weeks of enrichment. No bacteriophages were recovered from 12 samples of unenriched soil, from 40 samples of garden soil, or from 37 samples of dung soil.

Properties of Two Phages

The two bacteriophages isolated were designated *M. ranae* phage, MR_1, and *M. eos* phage, ME_1. The phages showed characteristic properties of other phages in that they reproduced on the susceptible host and lysed it to a high titre. *M. ranae* phage produced confluent lysis of its host on solid medium in a dilution of 10^{-5}, whereas *M. eos* phage lysed its host in a dilution of 10^{-4}. Both phages were inactivated between temperatures of 67–70° C. when held for 10 minutes. They were found to belong to different serological groups and maintained their potency when stored at 6° C. over a period of 6 months.

M. ranae phage produced circular plaques which varied from 1 to 2.5 mm. in diameter. Variation in types of plaques has been observed with this phage. The variations are similar to those of *M. phlei* phages (4). The plaques produced by *M. eos* phage are circular and pin-point in size, and no variations in size and shape have been observed.

Of 40 acid-fast strains tested, *M. ranae* phage was active for three strains. These were *M. ranae*, avirulent *M. tuberculosis* ATCC607, and *M. phlei*, Ph_1. *M. eos* phage was active for only two strains, *M. eos* and an unclassified acid-fast strain, 3158, which was later shown to be a *M. eos* strain. The latter phage, therefore, appears to be specific. Neither of these two phages was active for 10 human, 2 bovine, and 4 avian tubercle strains.

DISCUSSION

Of 80 samples of soil enriched with 10 different acid-fast micro-organisms, only three revealed phage. No phage was isolated for the pathogenic organisms by the enrichment technique or from 12 samples of unenriched soil. The specific enrichment of soil samples with the particular organism seems to facilitate the isolation of phages for saprophytic acid-fast organisms only.

This was found to be the case by other workers (1, 4, 6). The failure of tubercle bacilli strains to reveal the presence of phage may be attributed to the failure of these micro-organisms to multiply within the soil and the failure of the existing phages to adapt themselves to these strains of such a different nature.   In experiments to determine whether tubercle bacilli multiplied in the soil no evidence was obtained that such was the case. The fact that no phages were isolated from garden soil indicates that the nature of the soil is another factor which determines the success of isolation of phages.

The two phages isolated show characteristic bacteriophage activities. Their value in classification of acid-fast organisms is now being investigated.

SUMMARY

1. Bacteriophages for *M. ranae* and *M. eos* were isolated from samples of dung soil by specific enrichment with a heavy suspension of each organism.

2. The method of enrichment was not found suitable for isolating bacterio-phages for pathogenic acid-fast micro-organisms.

3. *M. ranae* phage was active for 3 strains and *M. eos* phage for 2 of 40 saprophytic strains tested. Neither was active for pathogenic tubercle bacilli. The two bacteriophages may prove of value in classification of the myco-bacterium genus.

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The Problem of Transport of Specimens for Culture of Gonococci

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CULTURE is generally recognized to be a more satisfactory diagnostic procedure than direct smear examination, particularly in the diagnosis of gonorrhoea in females. Where direct culture facilities are available in clinics or where the examining laboratory has direct access to clinical material, the culture of gonococci is a comparatively simple and dependable procedure. When such direct culture is not possible, an acute problem is immediately introduced by the notoriously irregular viability of the gonococcus outside the human body. Transport of ordinary swabs, even within a period of one hour, may be sufficient to destroy the organism. Many attempts have been made to overcome this difficulty, but the current conception of the situation is probably expressed in the most recent edition of the well-known Jordan-Burrows "Textbook of Bacteriology" (1949), which states that "no really satisfactory method of preserving specimens has been developed." This opinion is supported by many of the comments of Thayer, Peizer and Steffen, Hirschberg, and Coleman (1947) in a panel discussion on procedures recommended for the diagnosis of gonococcal infection (Carpenter, 1943).

A transport method, the basic principles and application of which were described by Stuart (1946) in Glasgow, Scotland, overcomes most of these objections and has the virtue of simplicity both in clinical application and in laboratory handling. Its value has been confirmed by at least three separate groups of workers, Le Minor, Le Minor and Combes (1949) from the Pasteur Institute, Paris, France; Wilkinson (1951), from the Whitechapel Clinic, London, and Alexander (1952), in Liverpool, England. The last worker makes the astonishing comment that this transport method is better than direct culture and that it produces the ideal gynecological specimen.

The following paper describes the rationale of the method, reviews its application to both British and Canadian conditions, and discusses certain modifications which have proved desirable under different conditions.

RATIONALE OF TRANSPORT METHOD

Throughout the years innumerable attempts have been made to devise a suitable method for the transport of viable gonococci from the patient to the

culture plate. The method by which the culture medium is inoculated and incubated in the clinic and the plates merely sent to the laboratory for investigation and interpretation can be set aside for the present. The more usual method, by which specimens are taken in the clinic and are then transferred to the laboratory, presents the real problem. A study of the literature shows that it has been approached in two distinct ways.

The first approach consists of introducing the swab into a fluid or semi-solid nutrient medium in an attempt to maintain the viability of the gonococcus during transport by encouraging its multiplication. Such media, and particularly the very rich and sometimes highly elaborate media employed, have the coincidental defect of encouraging the other organisms invariably present in such specimens to grow even better than gonococci. Some workers have attempted to discourage this by the use of various bacteriostatic dyes and antibiotics. Our experience and that of other workers (Reymann, 1944, Peizer and Steffen, 1947, Reyn, 1951) suggests that none of the inhibitory substances recommended—crystal violet, Nile Blue A, thallium acetate, tyrothricin—is sufficiently selective; in many instances the inhibition of some strains of the gonococcus by the "selective" agents is more than equal to the encouragement of their multiplication by the medium. The most obvious rationalization of this "give-and-take" approach is to abandon both nutrient materials and bacteriostatic agents and to use a non-nutrient transport vehicle.

The second approach stresses the importance of environment to maintain the viability of gonococci. Since both freezing specimens with dry ice (Wortmann *et al.*, 1941) and maintaining them at body temperature (Malcolm and Dolman, 1939) have been recommended, temperature alone seems relatively unimportant. All workers agree that drying of the specimen has to be prevented. Yet drying of bacteria is not necessarily harmful; indeed, it is the most generally accepted method of preserving them, as in freeze-drying techniques. These must, however, be carried out *in vacuo*. The comment by Rahn (1945) that the death of bacteria in drying is mainly due to oxidation clarifies this issue. Prevention of drying may well be an important environmental factor but prevention of oxidation is likely to be even more important.

The transport medium to be described has evolved out of the above conclusions. It is a soft agar medium which contains no nutrients but keeps the inserted swab moist during transport. Oxidation, experimentally found to be the most important factor in destroying gonococci, is prevented by the incorporation of sodium thioglycollate and the distribution of the medium in small bottles sealed by the rubber liner of the screw-cap. Various trivialities, such as the use of methylene blue as an indicator of the keeping quality of the medium, the addition of extra calcium and thus the necessity of using a compatible glycero-phosphate buffer, have each added a little to the value of the medium, but in every respect save one the medium and method have remained essentially unchanged since their introduction.

The one exception arose after the original method had been in clinical use for almost a year (Moffett, Young, and Stuart, 1948) and was due to certain observations made during routine experimental trials of a new batch of medium. Results with this batch were grossly inferior to those obtained

earlier and by elimination the defect was traced to a new batch of agar just
brought into use. That some inhibitory factor was involved was readily
established and the fact that it was found in no less than seven agars of dif-
ferent origin then available for tests showed that it was extremely common.
Indeed, the single batch of agar used up to this time was the only one in which
this inhibitor was found to be insignificant.

Coincidentally with this observation, Ley and Mueller (1946) identified a
factor in ordinary agar inhibitory to Neisseria. This factor was readily noticea-
ble only in the absence of nutrient material and was apparently a fatty acid.
The newly discovered variability in our gonococcal transport medium was
due to variable concentrations of a similar factor in batches of agar. This
inhibitor behaved in exactly the same fashion as the one described by Ley and
Mueller, who showed that it could be neutralized by corn starch and suggested
the addition of this substance to gonococcal culture media. Unfortunately the
incorporation of any nutrient material was completely opposed to one of the
basic principles of the transport medium. This necessitated a search for a
non-nutrient absorbent. The observations by Pelouze (1931) and Glass and
Kennett (1939) on the beneficial effect of the incorporation of charcoal into
Neisseria culture media, observations that remained unexplained, suggested
another line of approach based on the possibility that this charcoal effect was
due to absorption or neutralization of inhibitor. The later observation by
Klaesson (1946) that charcoal binds fatty acids finally added scientific support
to this hypothesis.

The Neutralization of Agar Inhibitors by Charcoal

Charcoal in various concentrations was introduced into the transport
medium and experimentally a significant improvement was observed in the
viability of gonococci (Table I). Various kinds of charcoal were used; material
commercially identified as animal charcoal, blood charcoal, activated charcoal
(Norit), and powdered graphite were all satisfactory when reduced to a fine
powder. Experimentally the extraction of an inhibitor from the agar medium
by charcoal could readily be demonstrated by incubating the medium with
fine charcoal powder incorporated and, after centrifuging out the charcoal,

TABLE I
IMPROVEMENT IN GONOCOCCAL VIABILITY BY INCORPORATION
OF CHARCOAL INTO TRANSPORT MEDIUM

Gonococcus strain	Routine medium (inhibitory agar)	Same medium plus charcoal	Immediate control*
A H.	15**	125	180
	100	150	
2125	0	100	200
	0	75	100
2201	4	75	100
	8	70	
2349	100	200	200
	50	175	

*Swab dipped in suspension of gonococci and cultured immediately.
**Approximate number of colonies of gonococci recovered from swab dipped in same sus-
pension and then left in transport medium 24 hours at room temperature.

finding that the medium had improved qualities for keeping gonococci alive. The inhibitor could be extracted from the charcoal by methanol. After evaporation of the solvent, reincorporation of a suspension of the residue into the original extracted medium produced an environment again unsatisfactory for the maintenance of gonococci (Table II). Results were always slightly irregular but the over-all effect was quite definite. The direct incorporation of charcoal

TABLE II

Number of Gonococci Recovered from Swabs Kept 24 Hours in Transport Medium and in Same Medium After Extraction and After Replacement of "Inhibitor"

Gonococcus strain	Routine medium (inhibitory agar)	Same medium (charcoal extraction)	Same medium "inhibitor" replaced
3413	5	75	16
	3	100	50
3833	0	44	0
	0	60	0
3475	13	16	0
	21	19	1
2922	0	50	50
	0	50	100

into the transport medium was not wholly satisfactory, because it absorbed the small amount of methylene blue included as a reduction indicator. This was unfortunate because some means of demonstrating the oxidation, and thus the unsuitability of an occasional bottle of transport medium, seemed important when transport bottles might be retained in clinics for uncontrolled time periods. Thus the only way to employ charcoal without using it in the transport medium was to incorporate it in the swab, a process quite easily done by dipping the prepared swabs into a suspension of charcoal, after which they were dried and sterilized. This proved successful. Not only did the charcoal swabs neutralize the inhibitor in "bad" agars but they also seemed to produce better results than previously obtained with the transport medium so far considered satisfactory. Actually in 150 experiments using 33 strains of gonococci this difference was maintained with only 3 exceptions in which the ordinary swab was as good as the charcoal swab. Table III exemplifies some of the experimental results. In this instance the immediate control was carried out by dipping ordinary and charcoal swabs in a suspension of gonococci,

TABLE III

Effect of Charcoal Swab on Recovery of Gonococci from Inhibitory and Apparently Non-inhibitory Transport Media

Swab	Bad Agar		Good Agar		Immediate Control	
	Ordinary	Charcoal	Ordinary	Charcoal	Ordinary	Charcoal
Gc. 3833	5	150	50	150	150	150
3475	50	150	5	75	100	200
3809	10	150	100	150	150	200
3845	50	200	150	200	200	200
4066	4	200	50	200	200	200

inserting them momentarily into bottles of transport medium to simulate the conditions of clinical use, then resuspending the bacteria on the swab in a small volume of peptone water to permit the immediate culture of a measured amount. Exactly the same procedure was followed with the "transport" swabs except that they were left in the transport medium for 24 hours at room temperature.

CURRENT USE OF TRANSPORT METHOD

This can be shown best by a brief outline of the preparation of the medium, the outfit as issued, the instructions sent with outfits and the treatment of swabs in the laboratory.

Transport Medium

Dissolve 6 gm. Bacto Agar in 1900 ml. distilled water. Add 2 ml. thioglycollic acid (Difco) and bring to approximately pH 7.2 with $\frac{N}{1}$ NaOH. Add 100 ml. solution of Na. glycerophosphate (20% w.v. in water) and 20 ml. solution of $CaCl_2$ (1% w.v. in water). Bring to pH 7.4 with $\frac{N}{1}$ HCl. Add 4 ml. methylene blue (0.1% in water). Dispense into $\frac{1}{4}$ oz. screw capped bottles, filling to capacity. Sterilize in flowing steam for 1 hour, afterwards screwing caps down tightly. Makes approximately 300 bottles.
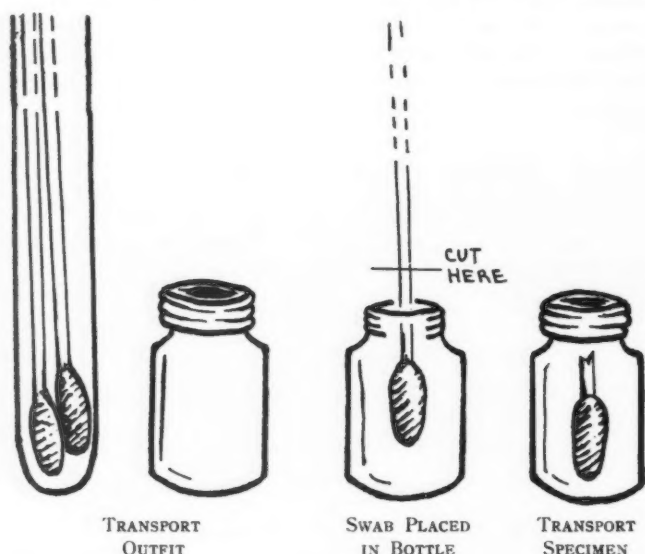
The Transport Outfit

This consists of a transport medium bottle and two swabs in the same container. The swabs are prepared as follows:

Good quality absorbent cotton and swab sticks are used. Prepared swabs are boiled in Sorensen's phosphate buffer solution pH 7.4 (probably unnecessary, but see Stuart, 1946) and immediately dipped in a 1% suspension of finely powdered charcoal in water. At present we use "activated charcoal" B.D.H. but "animal charcoal" (Cenco), "blood charcoal" B.D.H. and "Norit" have been used successfully. The swabs are then placed in cotton-plugged test-tubes (two in each tube), dried, and sterilized. (For certain clinics the stick of one swab is slightly charred close to the cotton. By mutual arrangement this simple procedure identifies the cervical from the urethral swab when both are inserted together in the same bottle.)

Instructions Sent With Outfits

Take the specimen and insert the swab into the upper third of the medium in the small bottle. Cut off the protruding portion of the swab stick with scissors and screw the lid on the bottle tightly. This usually forces the swab down slightly and centres it in the transport medium. Label, and return the bottle with the swab enclosed to the Provincial Laboratory *as soon as possible* with a request for Gc. culture. Should the specimen have to be retained in the office or clinic for some time before submission to the laboratory, it should be kept in the refrigerator.

The outfit and method of handling are illustrated by Figure 1.

TRANSPORT   SWAB PLACED   TRANSPORT
OUTFIT   IN BOTTLE   SPECIMEN

Laboratory Handling

In the laboratory the short swab stick in the transport bottle is seized with round-nosed artery forceps, the swab is rubbed firmly all over one quadrant of a culture plate, and the inoculation completed with a glass spreader in approximately the manner described by Morton and Lebermann (1944). Various culture media have been used, including Peizer, Difco Gc. Medium with Supplement B, starch-hæmoglobin agar and chocolate agar (McLeod *et al.*, 1934). Currently we use most commonly Difco Gc. Medium with a laboratory-prepared yeast extract supplement (Toshach, 1953). The selection of a final medium is perhaps best left to custom, prejudice or local convenience. In appropriate circumstances equivalent results can be obtained from each of the above.

Plates are incubated in a closed container in an atmosphere with increased CO_2. This has at various times been provided by the action of HCl on different carbonates, by compressed cylinder gas, and by the currently favoured candle technique. Most plates can be read at 24 hours, though a few require re-incubation for another day. All gonococci are identified by colonial appearance, oxidase-reaction and smear and are "confirmed" by failure of growth on ordinary agar and by fermentation of glucose, but not maltose.

After the original swab has been used to inoculate the culture media, an additional test can be carried out to determine the presence of living *Trichomonas vaginalis*. Material from the swab is suspended in a drop of peptone water, placed under a cover-glass on a slide, and examined microscopically with dry objectives and reduced illumination.

RESULTS

The experimental conclusions on the beneficial effect of charcoal swabs were soon supported by clinical observations. The figures (Table IV) come from Glasgow and show an early comparison of the results obtained by the use of plain cotton swabs in the transport medium as first prepared, using agar with little apparent inhibitor, and of charcoal impregnated swabs as used later with less satisfactory agars. Although the two sets of figures were obtained in different periods, they were based on material derived from the same clinics. The smear examinations were carried out completely independent of the laboratory and the same workers were involved throughout in both smear and culture examinations. Statistically the two groups of cases are similar, as can be determined from the percentage of positive smears. Taking these as an index of the quality of clinical sampling, we find the actual percentage difference between the two groups to be $10.66 - 7.59$ or 3.07, which is less than

TABLE IV

COMPARISON OF ORDINARY AND CHARCOAL SWAB TECHNIQUES
A—703 specimens on ordinary swabs
B—448 specimens on charcoal swabs

Smear	Culture	Percentage	
		A	B
+	+	7.82	6.47
+	0	2.84	1.12
0	+	5.41	11.16

twice the standard error of 1.76 and could arise by chance. The most evident difference in the two series lies in the percentage of positive results obtained by culture alone, 11.16 per cent. with charcoal swabs, 5.41 per cent. with ordinary swabs. By the chi-square test we find that P lies between 0.001 and 0.0001.Thus the apparent beneficial effect of the charcoal could occur by chance only once in between 1,000 and 10,000 experiments. This seems to make the observation of definite significance. Further comparison of such results using later figures has not been possible because of a developing statistical dissimilarity of populations concerned.

The total results obtained with charcoal swabs in Glasgow can be compared directly with those obtained with current techniques in Edmonton (Table V). The difference between the percentages of total positives identified by smear has no relation to the transport technique, but the interesting similarity in the corresponding percentages identified by culture does suggest that the transport technique has been of equivalent value in both areas.

The time factor is bound to have a considerable influence. In Glasgow specimens received later than 12 hours after being taken could almost always be received well within 24 hours, whereas in Alberta similar specimens reached the laboratory after approximately 24 hours or 48 hours of transport. Table VI gives a comparison of results in relation to time. From this table it can be seen that under 12 hours the Glasgow and Edmonton results are almost identical and it can be assumed from the evident progressive fall-off in culture results in relation to time that the difference in later results in the two areas is probably mainly dependent on this factor.

In both areas the transport method has proved satisfactory for the diagnosis of *Trichomonas* infection. In Glasgow the incidence of living trichomonads in transport specimens was approximately 45%, while in Edmonton it reached

TABLE V

COMPARISON OF RESULTS WITH SIMILAR TRANSPORT TECHNIQUES
IN GLASGOW AND EDMONTON

Glasgow: 1729 specimens		Edmonton: 2523 specimens	
Smear	Culture	Glasgow	Edmonton
+	+	80	142
+	0	25	35
0	+	167	142
0	0	1457	2204
Positive smears		105 (6%)	177 (7%)
Positive cultures		247 (14.3%)	284 (11.3%)
Total positives		272 (15.7%)	319 (12.6%)
Percentage of ⎱ by smear		38.6	55.5
total positives ⎰ by culture		90.8	89.0

only 13%. It is probable that this difference shows an actual difference in population infestation, but it may also be influenced by the time factor because in Glasgow a comparative investigation of the technique showed it to be unreliable after 24 hours.

TABLE VI

CULTURE RESULTS AFTER DIFFERENT PERIODS OF TRANSPORT

	Under 12 hours			Over 12 hours	
	Positive	Percentage of Possible		Positive	Percentage of Possible
Glasgow	62	94		185	90
Edmonton	158	95		126	82

	24 hours		48 hours or later	
	Positive	Percentage of Possible	Positive	Percentage of Possible
	113	83	13	77

DISCUSSION

The evidence presented in this paper shows clearly that the transport method described can be effective in areas of the world considerably different in climate, concentration of the population, and medical organization. This suggests that the method is probably of general application. It is almost independent of the necessity for specialized laboratory training of clinical workers and nursing staffs, and requires only intelligence and normal professional skill for its effective use. It can be applied to general practice as readily as to hospitals and organized clinics, and specimens can be sent to the laboratory by messenger or by mail. We are of the opinion that the high

temperature of sorting offices and mail cars in this country is slightly deleteri-
ous to specimens so exposed for prolonged periods. Similar reasoning has led
to the recommendation, based on experimental work in Alberta, that specimens
should be kept in the refrigerator before transport, rather than at room
temperature, as originally recommended in Scotland. Nevertheless our results
support the conclusions of other workers who have tested the method and
suggest that it is well over 90 per cent. effective under a transport time of
24 hours. Over 24 hours the viability of gonococci deteriorates progressively.
In exceptional instances the organisms can be recovered from swabs maintained
in the transport medium for longer than one week (from one specimen only
at 16 days), but we do not think the transport method is dependable more than
24 hours or useful more than three days. Up to this time a higher percentage
of positives will usually be recorded by culture than by smear and the comple-
mentary effect of the two examinations will still be valuable. We wish to stress
this complementary value because in our opinion it is the chief benefit from
the cultural investigation of gonorrhoea. It is extremely probable that the
combination of smear and culture, using the above transport method, achieves
diagnosis of practically one hundred per cent. of cases. Of course, this can only
be determined by clinical experience and judgment, but till now the only
clinical complaint we received in this connection was of our failure to culture
gonococci from a three-day-old specimen when the associated smear was
definitely positive but the clinical findings doubtful. The complementary
value of smear and culture is least evident in male gonorrhoea, and in our
experience few clinical workers find culture to produce any extra benefit in
such cases. We have, however, applied the transport method in certain in-
stances not described in this paper and have found it beneficial in certain cases
of chronic male gonorrhoea and prostatitis.

At least as important as the time factor in the culture of gonococci is the
presence of other bacteria in the specimen. Certain organisms always interfere
with the culture of Neisseria, perhaps by some specific action, but certainly
by competition or by spreading on nutrient plates. This is particularly true
of coliform bacilli and Proteus species. Occasional periods of prevalence of
such organisms have been regularly encountered but it has not been possible
to correlate these with any flaw in clinical technique nor to discover any
laboratory device for counteracting the mischief. The rich media, the soft
agar, and the humidity desirable for gonococcal culture are alike favorable to
such contaminants and only rarely is it possible, as in the elimination of a
troublesome spore-bearing contaminant by controlled humidity (Young,
Stuart, and Wilson, 1949), to control them in any way.

In the course of our cultural work we have isolated many Neisseria other
than gonococci but only in certain cases of meningococcal ophthalmia were
we able to refute smear diagnosis (Stuart and McWalter, 1948). Although
smear examination fails to detect a considerable number of positives, we have
always found a typical positive smear diagnostically reliable. Adventitious
Neisseria in culture are rarely confusing; only in one small group of cases, five
in number, did we encounter such organisms morphologically and, at least in
primary culture, culturally indistinguishable from true gonococci.

The efficacy of the transport method in the diagnosis of trichomonad infestation is not quite so high as its efficacy in gonococcal infection. Nevertheless it is easy to identify *Trichomonas* in the great majority of specimens from patients with a heavy infestation and thus probably from most patients in whom the finding is of any clinical significance. The simplicity of the method by which active living *Trichomonas* can be found in the laboratory up to 24 hours after the specimen is taken will impress many who recall the urgency usually associated with this examination.

We wish to emphasize that the described transport method utilizes a basic principle of bacteriology and therefore is likely to be applicable to many organisms other than Neisseria. We have used it for the recovery of *H. influenzae*, *D. pneumoniae*, *Str. pyogenes*, and *C. diphtheriae* with considerable success when specimens had to be sent from isolated areas over a time interval of three to five days. *H. pertussis* has also been recovered from a per-nasal swab in transport medium, but no opportunity has arisen for testing the efficacy of the medium more fully in this direction.

Summary

A convenient, simple and reliable method has been described for the transport of specimens for the culture of gonococci. The method is based on three main principles:

(a) The use of swabs treated with charcoal to neutralize an inhibitory factor present in the agar used in the transport medium.

(b) The prevention of oxidation and desiccation of material on these swabs to maintain gonococcal viability.

(c) The absence of nutrient substance in the transport vehicle to prevent undesirable multiplication of adventitious organisms.

Excellent results have been obtained both in Scotland and Alberta by the application of this method to the diagnosis of female gonorrhoea; in both areas the positives detected have practically doubled the number diagnosed by smear examination alone. The method will allow the isolation of gonococci from approximately 90 per cent. of cases when the transport period is under 24 hours; over that time the method is still useful up to three days, but in this connection the complementary value of smear and culture has been emphasized.

The transport method also maintains the viability of *Trichomonas vaginalis* up to 24 hours and thus detection of this parasite can be made a convenient laboratory investigation rather than an urgent clinical procedure.

The transport method can be applied to other bacteria and it is suggested that it may be of general applicability to bacteriological diagnosis when specimens are likely to be considerably delayed in reaching the laboratory.

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EFFORTS TOWARDS THE PREVENTION OF POLIOMYELITIS

THE data of Dr. William McD. Hammon and his associates relating to observations on approximately 55,000 children and presented in detail in 1952, provided the evidence on which the use of gamma globulin in the prevention or modification of poliomyelitis is based. The observations of Dr. Hammon, using gamma globulin prepared for measles prophylaxis by the American Red Cross, were made possible by the National Foundation for Infantile Paralysis, Inc., New York (the American "March of Dimes"). Dr. Hammon's summary stated: "During the first week after injection, there was no significant reduction in the number of cases in the group receiving gamma globulin, but the severity of paralysis appeared to be modified. From the second to the fifth weeks, highly significant protection was demonstrated. After the fifth week this was less evident."

In planning for the distribution of large quantities of gamma globulin by the National Foundation in 1953, arrangements were made for the mass administration of gamma globulin in communities as soon as possible after the occurrence of poliomyelitis in the area. The first trial was made in Montgomery, Alabama, where 30,000 children received gamma globulin. The results reported by Dr. D. G. Gill were encouraging. Similar mass administration was tried in several other cities. In attempting to appraise the results, it has been found that the administration of gamma globulin was too limited or too late to be effective. Further observations in centres where the conditions of trial are satisfactory must be made in order to determine if mass administration is of value in cutting short an epidemic. In considering the possible value of gamma globulin in the prevention of poliomyelitis, it has been appreciated from the commencement that its use in family contacts was hardly justified, since second cases in families usually occurred within a week and gamma globulin did not seem to be effective until some days after its administration. However, the availability of globulin resulted in its widespread use in contacts. Analysis of such data as are available would seem to indicate that gamma globulin has little or no value in reducing the incidence or the extent of paralysis in contacts. It is hoped that, during 1954, trials will be conducted which will give a specific answer to this question. The administration of gamma globulin to pregnant women is recommended, since globulin may be administered as a preventive of poliomyelitis and repeated in a month's time, when necessary.

In Canada last year approximately 30,000 vials of gamma globulin were

made available for use by the Provincial Departments of Health. This was a relatively small amount, but facilities for the production of the serum in quantity did not exist in Canada in the fall of 1952, when Dr. Hammon's results were announced. Through the co-operation of the Provincial Departments of Health and the Federal Department of Health, financial assistance was given to the Connaught Medical Research Laboratories, to make possible the establishing of production within a few months. As a donation of one pint of human blood is required for one dose of gamma globulin, the problem of supplying the serum in quantity is one of great magnitude. In this undertaking, the Canadian Red Cross Society made possible the collection of blood from across Canada. In the meantime, the Laboratories fortunately had on hand a supply of human blood serum which was not satisfactory for clinical use. This serum was used for the immediate production of gamma globulin, permitting the Canadian Red Cross Society to plan for the increased donations of blood. The objective which has been set for 1954 in Canada is 100,000 vials of this serum. For this, 150,000 additional blood donations will be necessary.

It is appreciated that the large-scale use of gamma globulin does not provide an answer to the problem of the prevention of poliomyelitis. The ultimate goal must be the development of means to confer immunity by vaccination. Interest today centres in the trial of a preventive vaccine introduced by Dr. Jonas E. Salk, of the University of Pittsburgh. This trial is being made under the sponsorship of the National Foundation for Infantile Paralysis. The vaccine is based on the initial work of Dr. John F. Enders and his colleagues, who in 1949 demonstrated the growth of poliomyelitis virus in culture fluids containing pieces of monkey testicle. At the University of Toronto, in the Connaught Medical Research Laboratories, a synthetic medium (known as no. 199) for the growth of animal cells, which was introduced by Morton, Morgan and Parker, is being used and cultures are being successfully made, using monkey kidney tissue. The technique which is employed in the preparation of poliomyelitis culture fluids in quantity was developed in the Connaught Medical Research Laboratories and presented in a paper published in August, 1953.*

The culture fluids are used in the preparation of the vaccine, the viruses of poliomyelitis being inactivated by formalin. A description of the method of preparation has been published in several articles by Dr. Salk. Every precaution is being taken to assure the safety of the vaccine. Before its distribution, a series of tests will be made by the manufacturer, by Dr. Salk, and in the laboratories of the National Institutes of Health, Washington. To date, more than 700 children have received this vaccine and serological tests to determine their immunological responses have been conducted on a group who received it a year ago. To determine the possible value of the vaccine will require observations in hundreds of thousands of children, in all parts of the United States. The attack rate of poliomyelitis varies greatly in different sections of the country, and large groups will be necessary to obtain figures that are statistically significant.

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